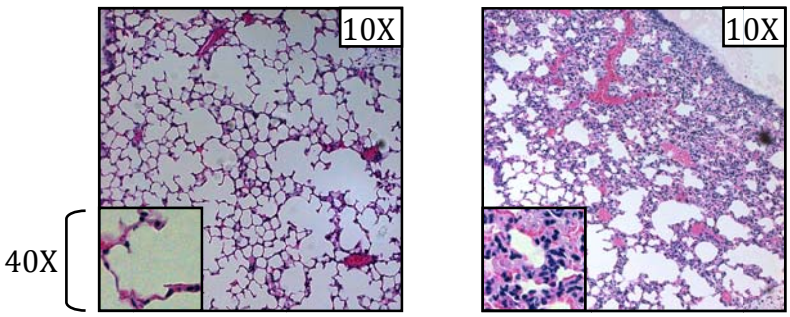
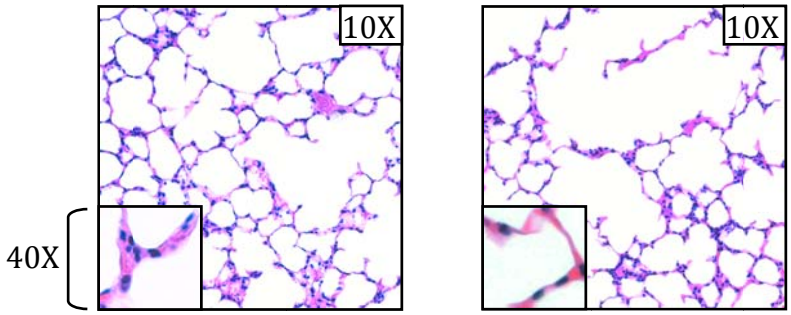
Supplemental Digital Content 1

A



Genotype/Sample ID =	B6 wild type left lung	B6 wild type left lung
Ischemia time =	Sham (30 minutes)	30 minutes
Reperfusion time =	Sham (3 h)	3 h

B

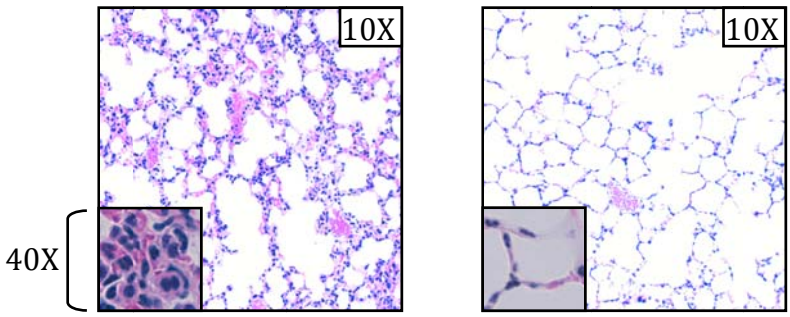


Genotype/Sample ID =	B6 wild type left lung	B6 wild type left lung
Ischemia time =	Sham (30 minutes)	30 minutes
Reperfusion time =	Sham (1 h)	1 h

Fig. 1. Shortened ischemia time also results in neutrophil infiltration that begins between 1 h and 3 h following reperfusion.

(A) Histology from C57/BL6 wild type mice exposed to shorter ischemia time (30min) followed by reperfusion (3h) compared to matched mice exposed to the sham procedure (Hematoxylin and Eosin staining, 10X and 40X magnifications). Images are representative of 3-4 independent surgeries.

(B) Histology from C57/BL6 wild type mice at time points used in Figure 1B from text (30 minutes ischemia, 1 h reperfusion versus sham surgery).



Genotype/Sample ID =	B6 wild type left lung	B6 TLR4 -/- left lung
Ischemia time =	30 minutes	30 minutes
Reperfusion time =	3 h	3 h

Fig. 2. Toll like Receptor-4 (TLR4) -/- mice on C57/BL6 background are also protected against ventilated lung Ischemia Reperfusion (I/R) injury.

C57BL/6 background TLR4 -/- mice and wild type matched controls underwent the lung I/R surgery followed by 3h of reperfusion following which left lower lung samples were analyzed by Hematoxylin and Eosin histopathology.

Images are representative of 3 independent experiments.

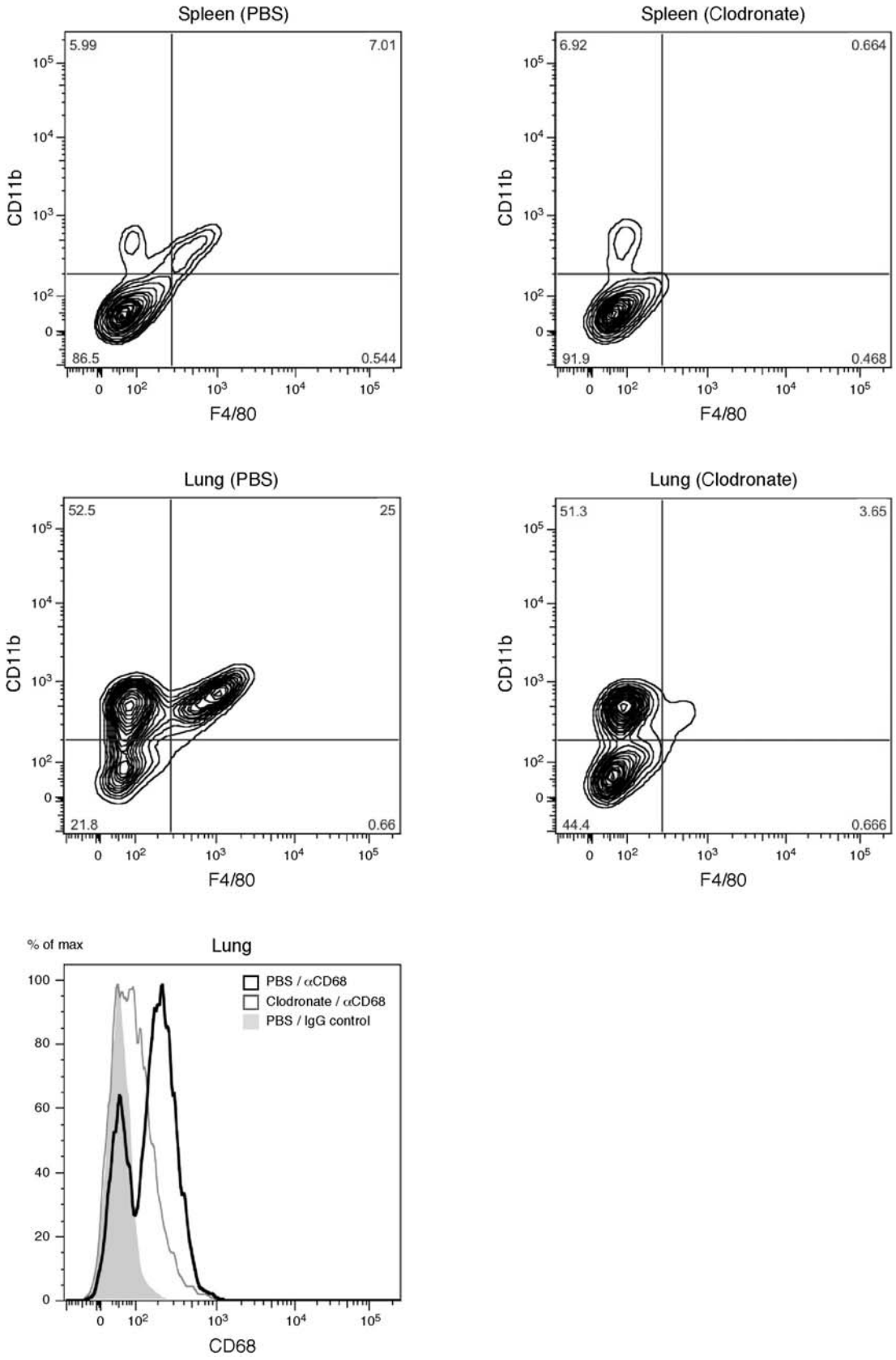
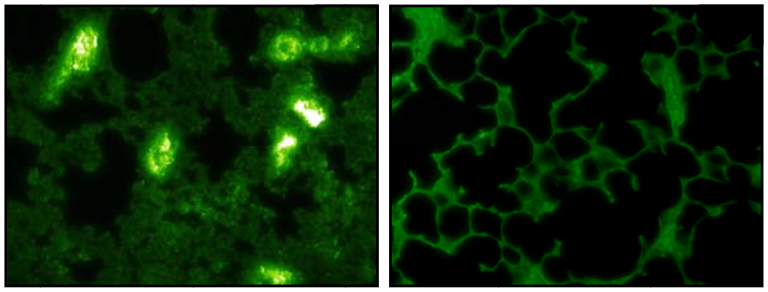


Fig. 3. Systemic clodronate liposome treatment results in depletion of splenic macrophages and pulmonary alveolar and interstitial macrophages.

Mice that were either pretreated with phosphate buffered saline (PBS) carrier (intravenous) or with clodronate liposomes (intravenous) were assessed by flow cytometry for macrophage depletion in their digested lungs and spleen using F4/80, CD11b and CD68 antibodies as indicated. Cells were permeabilized for CD68 staining (marking alveolar macrophages).

Data are representative of 2-3 independent experiments.

IgG – Immunoglobulin G isotype control antibody



Genotype/Sample ID =	CD11c-DTR left lung IR	CD11c-DTR left lung IR
Pretreatment =	PBS (IP)	DTx (IP)
Ischemia time =	30 minutes	30 minutes
Reperfusion time =	3 h	3 h

Fig. 4. immunofluorescence verifies CD11c+ cell depletion in CD11c-DTR mouse lungs treated with diphtheria Toxin (DTx).

Verification of alveolar macrophage (CD11c+ cell) depletion following treatment with DTx by immunofluorescence. CD11c-DTR mice with enhanced green fluorescent protein (EGFP) driven by the CD11c promoter and treated with DTx or phosphate buffered saline (PBS) (IP = intraperitoneally).